GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: September 5, 1980

Project Title: Evaluation of the Synthetic Potential of Enzymatic Catalysts for Oxyfunctionalization and Oxidation of Organic Compounds

Project No: G-33-667

Project Director: Dr. Sheldon W. May

Sponsor: American Chemical Society; The Petroleum Research Fund

Agreement Period: From 9/1/80 Until 8/31/82 8/31/83

Type Agreement: PRF Grant No. 12605-AC1

Amount: $30,000

Reports Required: Annual Comprehensive Reports

Sponsor Contact Person (s):

Technical Matters

Contractual Matters
(thru OCA)

Dr. Justin W. Collat
Program Administrator
Petroleum Research Fund
American Chemical Society
1155 Sixteenth Street, N. W.
Washington, D. C. 20036

NOTE: Continuation of G-33-626

Defense Priority Rating: N/A

Assigned to: Chemistry (School/Laboratory)

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GA 3 (3/76)

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date 8-31-88

Project No. G-33-667 School/[redacted] Chemistry

Includes Subproject No.(s) N/A

Project Director(s) Dr. Sheldon W. May GTRC/[redacted]

Sponsor American Chemical Society; The Petroleum Research Fund

Title Evaluation of the Synthetic Potential of Enzymatic Catalysts for Oxyfunctionalization and Oxidation of Organic Compounds

Effective Completion Date: 8-31-86 (Performance) N/A (Reports

Grant/Contract Closeout Actions Remaining: No further reporting required

- [X] None
- [ ] Final Invoice or Copy of Last Invoice Serving as Final
- [ ] Release and Assignment
- [ ] Final Report of Inventions and/or Subcontract:
  Patent and Subcontract Questionnaire sent to Project Director [ ]
- [ ] Govt. Property Inventory & Related Certificate
- [ ] Classified Material Certificate
- [ ] Other

Continues Project No. G-33-626 Continued by Project No.

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G 33-667

THE PETROLEUM RESEARCH FUND

REPORT ON ACTIVITY ASSISTED BY

GRANT, PRF # 12605

Page 1 of 2 pages.

PREPARED BY

Sheldon W. May

Date September 30, 1981

Please refer to instructions.

Fill in information requested above for each page.

The report heading, narrative, and all drawings must be prepared within the box.

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12605-AC1 Evaluation of the Synthetic Potential of Enzymatic Catalysts for Oxyfunctionalization and Oxidation of Organic Compounds

Sheldon W. May. Georgia Institute of Technology

During the first year of this project our objective was to identify enzymatic catalysts which carry out oxyfunctionalization reactions of particular interest from a synthetic point of view, and do so with simple organic substrates. We have now identified, and are characterizing and developing for possible synthetic use, oxygenase systems which carry out oxygenation of heteroatoms, oxygenative ketonization of alcohols, and epoxidation of olefins. All of these reactions represent basic, synthetically important transformations. In all cases, the reactions are highly regio- and stereoselective, and have been carried out on a preparative scale. Our results have established for the first time that enzymes previously considered to be simple "hydroxylases" readily carry out these three reactions types.

Some specific examples of these reaction types are as follows. Working with the copper-containing monooxygenase, dopamine-β-hydroxylase, we have demonstrated for the first time sulfoxidation by an enzyme heretofore considered to be a "hydroxylase". Sulfoxidation exhibits all characteristics diagnostic of a monooxygenase reaction, and is stereospecific. The stereochemistry of oxygen attack is fully consistent with that established for methylene hydroxylation by this enzyme. Furthermore, detailed kinetic experiments have established that sulfoxidation proceeds much more readily than hydroxylation for comparable substrates. Preparative scale production of essentially optically pure sulfoxides has been easily accomplished, even with crude enzyme preparations.

Turning to ketone production, we have utilized two approaches to the enzymatic generation of ketones with controlled stereo- and regiospecificity. Firstly, we have demonstrated that enzymatic hydroxylation of the enantiomers of normal products can generate ketones via an oxygenase pathway, a process which is unprecedented and totally distinct from the familiar alcohol dehydrogenase reaction. Again with dopamine-β-hydroxylase, we have demonstrated that ketonization via this pathway is kinetically facile, and preparative scale experiments easily produce iso-

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lated yields in the mg/ml range, even with crude enzyme preparations. We are currently investigating whether this approach to stereoselective ketonization will be applicable to other oxygenases. Our second approach to ketonization has been the identification, isolation, and purification of a secondary alcohol dehydrogenase which produces ketones from a variety of straight-chain and alicyclic secondary alcohols of moderate chain length, while primary alcohols are unreactive. Our dehydrogenase has a much broader specificity than similar enzymes recently described in the literature. Preparative scale generation of ketones using both crude and purified enzyme preparations have again been successfully demonstrated in this case.

G-33-667

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G-33-667/May

PRF 8/78 - 1

12605-AC1 Evaluation of the Synthetic Potential of Enzymatic Catalysts for Oxy-functionalization and Oxidation of Organic Compounds.

Sheldon W. May. Georgia Institute of Technology

Our objective in this project is to identify enzymatic catalysts which carry out oxyfunctionalization reactions of particular interest from a synthetic point of view, and do so with simple organic substrates. We have now identified, and are characterizing and developing for possible synthetic use, oxygenase systems which carry out oxygenation of heteroatoms, oxygenative ketonization of alcohols, and epoxidation of olefins. All of these reactions represent basic, synthetically important transformations. In all cases, the reactions are highly regio- and stereoselective, and have been carried out on a preparative scale. Our results have established for the first time that enzymes previously considered to be imple "hydroxylases" readily carry out these three reactions types.

Some specific examples of these reaction types are as follows. Working with the copper-containing monooxygenase, dopamine-β-hydroxylase, we have demonstrated for the first time sulfoxidation by an enzyme heretofore considered to be a "hydroxylase". Sulfoxidation exhibits all characteristics diagnostic of a monooxygenase reaction, and is stereospecific. The stereochemistry of oxygen attack is fully consistent with that established for methylene hydroxylation by this enzyme. Furthermore, detailed kinetic experiments have established that sulfoxidation proceeds much more readily than hydroxylation for comparable substrates. Preparative scale production of essentially optically pure sulfoxides has been easily accomplished, even with crude enzyme preparations.

Within the past few months, we have extended these studies to other heteroatoms and have established that enzymatic oxygenation also occurs readily in these cases with Dopamine-β-Hydroxylase, but not with the non-heme iron "expoxidase/hydroxylase" from P.-Oleovorans. The mechanistic basis for this difference in reactivity is currently under

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investigation.

Enzymatic production of aldehydes and ketones has also been an area of interest to us. We have now established that aldehydes can be produced from simple terminal olefins by our non-heme iron monoxygenase. We view the mechanism of this reaction as involving a radical intermediate formed from initial attack of the activated iron-oxo (formally $Fe^{V}=O$) species on the terminal carbon of the of the oletin, followed by H atom migration and collapse to the aldehyde product. Evidence for this mechanism comes from D migration studies, and work with F-substituted compounds. Turning to ketone production, we are developing two approaches to the generation of ketones with controlled stereo- and regiospecificity. Firstly, we have demonstrated that enzymatic hydroxylation of the enantiomers of normal products can generate ketones via an oxygenase pathway, a process which is unprecedented and totally distinct from the familiar alcohol dehydrogenase reaction. Again with dopamine-β-hydroxylase, we have demonstrated that ketonization via this pathway is kinetically facile, and preparative scale experiments easily produce isolated yeilds in the mg/ml range, even with crude enzyme preparations. We are currently investigating whether this approach to stereoselective ketonization will be applicable to other oxygenases. Our second approach to ketonization has been the identification, isolation, and purification of a secondary alcohol dehydrogenase which produces ketones from a variety of straight-chain and alicyclic secondary alcohols of moderate chain length, while primary alcohols are unreactive. Our dehydrogenase has a much broader specificity than similar enzymes recently described in the literature. Preparative scale generation of ketones using both crude and purified enzyme preparations have again been successfully demonstrated in this case.

G33-667 MAY Subj Yrp + ACS By S. MAY 10/6/83 G-33-667

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Our objective in this project is to identify enzymatic catalysts which carry out oxyfunctionalization reactions of particular interest from a synthetic point of view, and do so with simple organic substrates. We have now identified, and are characterizing and developing for possible synthetic use, oxygenase systems which carry out oxygenation of heteroatoms, oxygenative ketonization of alcohols, and epoxidation of olefins. All of these reactions represent basic, synthetically important transformations. In all cases, the reactions are highly regio- and stereoselective, and have been carried out on a preparative scale. Our results have established for the first time that enzymes previously considered to be imple "hydroxylases" readily carry out these three reactions types.

In a specific example of this approach with the copper monooxygenase dopamine-B-hydroxylase, we demonstrated for the first time sulfoxidation by an enzyme heretofore considered to be a "hydroxylase". Sulfoxidation exhibits all characteristics diagnostic of a monooxygenase reaction, and is stereospecific. The stereochemistry of oxygen attack is fully consistent with that established for methylene hydroxylation by this enzyme. Furthermore, detailed kinetic experiments have established that sulfoxidation proceeds much more readily than hydroxylation for comparable substrates. Preparative scale production of essentially optically pure sulfoxides has been easily accomplished, even with crude enzyme preparations.

Such studies are now being extended in several ways. First, we are examining other enzymes heretofore considered "specific" hydroxylases for the facility and stereospecificity with which they may be able to carry out effect sulfoxidation of

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appropriate substrates. Secondly, we are examining other heteroatoms in order to scope the limits of heteroatom oxygenation reactions which can be effected with these enzymatic catalysts. Finally, using the approaches of physical organic chemistry, we are attempting to develop a mechanistic rationale for differences we observe in the abilities of oxygenases of different classes to carry out heteroatom oxygenation. Specific examples of these approaches are current investigations of Se oxygenation by dopamine-B-hydroxylase, and S oxygenation by the non-heme-iron dependent epoxidase of P. oleovorans.

Finally, we have initiated studies aimed at exploiting our ability to generate in high yield chiral epoxides from simple olefins with our enzymatic catalysts. Such chiral epoxides are attractive from a synthetic viewpoint, since they can be opened readily to give chiral synthons not readily obtainable by purely chemical means. Thus, through a combination of enzymatic and chemical steps, synthetically-important species may be generated from simple olefin precursors.